AD_____

GRANT NUMBER DAMD17-94-J-4357

TITLE: p53 Mutation Profiles in Premenopausal Versus Postmenopausal Breast Cancer in a Stationary Population

PRINCIPAL INVESTIGATOR: Bao-Fa Yu, M.D.

CONTRACTING ORGANIZATION: Salk Institute for Biological Studies
La Jolla, California 92037-1099

REPORT DATE: August 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1997	Annual (1 Sep 96 - 31 Aug 97)

4. TITLE AND SUBTITLE p53 Mutation Profiles in Premenopausal Versus Postmenopausal Breast Cancer in a Stationary Population

5. FUNDING NUMBERS
DAMD17-94-J-4357

6. AUTHOR(S)
Bao-Fa Yu, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Salk Institute for Biological Studies
La Jolla, California 92037-1099

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Abstract: There is a difference in the profiles of p53 mutation in pre and post menopausal breast cancers, and between the stationary polpulation and mixed population. This preliminary results is help to understand why BC incidence in US is 4 to 10 times higher than that in China at the molecular level and compare the incidence between the pre and post menopausal BC and between the stationary population of Shandong, China and mixed population of California, USA. These studies emphasize a study gene markers in groups of BC patients, divided into specific categories.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Humans, Anatomical Samples, p53, Mutations, Loh, Breast Cancer, Cancer Molecular Epideiology	16
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

__X__ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

__X__ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

__X__ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Barbara W 4-20-98

PI - Signature Date

(4) Table of contents:

(5) Introduction:

(6) Body:

(7) Conclusion:

(8) References:

(9) Appendix:

(5) Introduction:

The breast cancer incidence in America is 4.6 times higher than in Shanghai, China. The divergence in the curves of age-specific incidence of breast cancer is most dramatic in the post-menopausal years where the American have ten-fold higher rates than Chinese in Shanghai. No previous reports in the literature was found why there is a siginificant difference of breast cancer incidence between the America and China. We are trying to simplify the model of research and choose breast cancers from Shandong province of China, where the population has been stationary over many generations and California which has a mixed population. The inactivation of p53 tumor suppressor gene appears to play an important role in human breast cancer. The objective of this proposal is understand the difference at molecular level and compare the incidence of p53 mutations in breast cancers of premenopausal versus postmenopausal women between stationary and mixed population noted above.

Preliminary observations from our laboratory have shown that a p53 mutation is often located in exon 5 for postmenopausal breast cancer and located in different exons for premenopausal breast cancer in the stationary Shandong population. In contrast, the p53 mutation is often located in exon 5 for premenopausal breast cancer and located in different exons for postmenopausal breast cancers in the mixed population of California. These studies emphasize a study of the gene markers in groups of breast cancer patients, divided into specific categories. In this proposal, we aim to further test the hypothesis that p53 mutation patterns are different between the pre- and post-menopausal breast cancers, and between the stationary and mixed population desired. Using DNA extracted from paraffin embedded normal and tumor tissue, we will perform p53 mutation analysis by single stranded conformation polymorphism followed by nucleotide sequencing. If additional mutations are found, we will look for the presence of specific hot spots or the frequent occurrence of a specific type of mutation. The p53 gene mutation patterns may give us some clues whether diet, environmental exposure, or genetic factors lead to the higher than normal incidence of breast cancer in the California population which is useful for prevention and therapy of breast cancer.

(6) Body:

Background: The etiology of breast cancer (BC) involves a complex interplay of the genetic, hormonal and dietary factors that are superimposed on the physiological status of the host. Many gene alterations have been detected in BC, including amplification of oncogenes and mutation of tumor suppressor (TS) genes (1, 2, 38). The alterations of TS gene p53 was frequently detected in BC (40%) which associated with Li-Fraumeni syndrome (3). However, there is a possibility that exposure to mutagens and genetic predisposition influence p53 mutagenesis to a greater extent in some locales more than others (4), so that p53 may be a useful epidemiological tool for identification of mutagen and genetic factors to BC.

Epidemiological studies on BC in USA and China: It was reported that comparative epidemiology of BC in Shanghai, China versus the United States in 1991 (5), which showed the disparity in the incidence of BC concomitant with dramatic differences in diet between the U.S. and China. BC incidence is 4.6 times higher in Americans than in Chinese from Shanghai (5). The divergence in the curves of age-specific incidence of BC between these two groups is most dramatic in the post-menopausal years where Americans have ten-fold higher rates than the Chinese of Shanghai. Overall cancer risk in China is represented solely by data from Shanghai, which is unique and non-representive of China in many ways. In addition, it is highly industrialized compared to the remainder of the country, and the mortality due to BC is 20-30% higher in the population of Shanghai than the entire Chinese population. There are no reports comparing BC at the molecular level, such as studying the TS gene p53, between Americans and Chinese, and between pre- and postmenopausal women. To simplify the model of research, we choose BC from the small, colonized, Shandong province of China, where the population is stationary over many generations (Figure 1). This data will help us to identify the elements of dietary, environmental, and genetic contributions to the development of BC.

p53 gene in BC: By the age of 75 approximately 10% of all U.S. women will have developed BC. There is evidence for a genetic contribution to the risk of developing BC as well as an association with modern affluence (i.e. diet and alcohol consumption). In addition, the influence of reproductive factors supports a hormonal role in the etiology of the BC (6, 7). BC is, however, a heterogeneous disease (8), and for this reason linkage studies to pinpoint a genetic locus or loci responsible for the inherited susceptibility have been set with difficulties.

BRCA1 gene in BC: It is believed that htere is one TS gene on chromosome 17q for BC, one candidate is BRCA1 gene which genetically linked to the development

of some familial BC and located at 17q21 (38, 39, 40). Mutation were detected in 3 of 32 BC which was germline alterations and occurred in early-onset cancer, it suggusts that mutation of BRCA1 may not be cririca1 in th edevelopment of the majority of breast that arise in the absence of a mutant germline allele (41). There are many evidence for involvement of BRCA1 in sporadic and familial BC (42, 43) but it still can not neglect the important of p53 gene in the BC.

Loss of heterozygosity in BC: Studies on the loss of heterozygosity (LOH) in sporadic BC have shown loss of genetic material at a number of loci including 1q, 3p, 6q, 17p and 18q in greater than 50%, and at 1p, 7q, 8q, 9q, 11q, 13q, 15q, 17q, and 22q in more than 30% of tumors (9, 10) suggesting the possible involvement of a number of TS genes. Moreover, several oncogenes (*myc*, *neu*/HER-2 and *int* 2) have been implicated in the development of the disease (11). However, one gene which is clearly involved in the development of both sporadic and some hereditary BC is p53 (1-17), a gene which in its unmutated form behaves as a TS gene, but which in tumors is present in several mutant forms.

Mutation of p53 gene in BC: Mutation of the p53 gene is the most common genetic alteration in BC (18, 19) and has been found at numerous sites in more than 100 of the 393 amino acids that comprise the protein of p53 (20, 21). Such a variety of mutations at a large number of sites has permitted analysis for significant differences in the mutational spectra among different cancers. The nature of molecular change is often specific for a given mutagen; for example, UV light predominantly causes cyclobutane dimers (22), while N-methyl-nitrosourea produces G to A transition (23), so that a study of the nature of the mutations in p53 may provide evidence as to the nature of the mutagen (s) involved. The data from many studies have shown that more than 40% of primary BC containing mutations in the p53 gene. It was reported that 60% of sporadic BC have mutations in the p53 gene, but this data is controversial. Most mutations were distributed within exon 5, 6, and 7, whereas small deletions were distributed evenly from exon 4 to 8. Three region showing mutations most frequently were codon 175 in exon 5, codon 214-220 in exon 6 and 234-251 in exon 7 (16), and a preponderance of mutations was found at codons 175, 194, 273, and 280, but no particular mutational hot spots were identified (25). In sporadic BC, the increased frequency of GC-TA transversions, together with a very high incidence of guanosine mutations in the non-transcribed strand of the p53 gene, lead to the conclusion that exogenous carcinogens have an etiological role in these tumors. Carcinogenic hormones are generally inefficient in the production of point mutations at the gene level (24) but increased estrogen levels are known to promote cell growth and may indirectly increase the incidence of mutation. In a

recent study, p53 mutations were found to occur more frequently in BC occurring in premenopausal patients, especially in the ages of 26-30 years old (75%) (26). The mutations of p53 gene seemed to play an important role in the development of BC in these young patients. Otherwise, such higher incidence of p53 mutations might be explained by the fact that all of these young patients carried Grade 3 tumors, in which p53 mutations are frequent (26). There are no reports on comparing the abnormal p53 gene between the premenopausal BC (in patients less than 35 years old) and postmenopausal BC (in patients more than 70 years old) in the literature, and no reports on comparing p53 mutation patterns between BC in stationary and mixed populations.

p53 gene mutation in a familial BC: Families with striking histories of BC and other neoplasms suggest a new familial cancer syndrome of diverse tumors, referred to as Li-Fraumeni Syndrome (LFS) (27). Prospective studies have confirmed the high risk in family members of the tumors types that comprise LFS. A two-step mutation model was proposed. The model is based on the premise that most cancers are derived from a single cell and that at least two mutational events are required for the development of cancer. In hereditary cancers, the first mutational event is inherited and present in all cells of an individual at birth and can be transmitted through the germ cells, the second event is somatic. In sporadic cancers, both mutations are somatic (29). The alterations of the p53 gene occur not only as somatic mutations in human cancers, but also as germ line p53 mutation in some cancer-prone families. All cells in individuals with LFS also have a single wild-type p53 allele which provides an opportunity to compare the effects of p53 inactivation on the development of cancer in different tissues (28). Germ-line p53 mutations in the LFS are mainly CG-TA transitions at CpG dinucleotides. These may be naturally occurring endogenous events. Three factors leading to high risk of BC are the family history, early onset (young age), and bilaterality. Patients with diagnosis made during the premenopausal period may have an inherited genetic basis for BC. Our preliminary studies have shown that p53 mutations in BC with family cancer occur much more frequent.

Pattern of p53 gene mutations in BC of USA: The patterns of specific mutations within the p53 gene differ (4). These differences raise the possibility that p53 gene may be a useful epidemiological tool as a gene marker for the identification of mutagens and genetic factors that contribute to cancers, especially for BC (20). In one study (4), the tumors of 31.8% of 43 patients in the Midwestern United States with a age of 65 years contained mutations. There were five microdeletions ranging from 1 to 14 base pairs, three of which produced frame shifts and two of which maintained the reading frame. one had a single-base

substitution generating a stop codon and another had a single-base substitution generating a splice junction. Each amino acid substitution occurred at residues that are identical in the p53 gene of seven species. The data hint that mutations in the p53 gene are somewhat more likely in stage III and IV tumors. The pattern of mutations in U.S. population differs from that reported by other investigators. Of the 14 mutations found in our study, five of nine base substitutions occurred at CpG sites and five microdeletions. Of 31 mutations in human BC and BC cell lines published by other investigators, only two transitions at CpG and three microdeletions have been reported. The data (4) strongly suggests that the patterns of p53 mutations could serve as an epidemiological tool for the study of BC.

Methods: The experimental design is built on PCR-based methodologies due to two limitations: 1). the availability of limited, small amounts of tumor tissue/sections and 2) working with partially degraded DNA from paraffin-embedded samples. Most of the BC tissues are available as fixed tissues embedded in paraffin blocks and some are fresh tissues. Genetic analysis of human tumors is often complicated by the presence of varying percentages of stromal cell contamination (30). Therefore, we have selected techniques for the analysis of p53 gene that have a sensitivity high enough to detect gene alterations even in the presence of 50-75% normal cells.

Tissue Processing: To determine the tumor/normal composition of the tissue, the first section is deparaffinized, stained with hematoxylin and eosin, and examined immediately. If the section consists of more than 50% normal cells, the areas of the section carrying the normal cells is marked with a pen. The corresponding area is gently scraped off the remaining sections. The last section, scraped for enrichment is stained and examined, in each of the tissue blocks before and after enrichment is noted. Wherever possible, the "normal tissue" that is scraped away will be saved as a source of constitutional DNA from the patients. Again, the extent of contamination of this tissue with tumor cells will be documented. Our pathologist researcher, Dr. Nissi Varki (UCSD), will perform all of the examinations, so that there is uniformity in diagnosis and evaluation of the tissues.

PCR-procedure: This was previously performed on DNA extracted from the paraffin embedded section using a 3-day extraction procedure utilizing detergents and enzyme digestion. The following modifications have resulted in better quality DNA. DNA is extracted from 8-10 micro paraffin section of breast cancer first with xylene. The pellet is re-extracted twice with 95% ethanol (31). The resultant pellet is dried in a speed-vac, resuspended in PCR buffer, heated to

95°C for 5 minutes, and aliquoted into several tubes for PCR analysis. The polymerase chain reaction is performed as described previously (32).

Analysis of mutations in the p53 gene: The vast majority of mutations in the p53 gene in breast cancers have been located in exons 5 to 9 (25, 26). The SSCP approach we have been using with DNA from both tissue and paraffin embedded sections is essentially that described by Gaidano et. al. (31). Using intron-sequence primers for each exon, exons 5-9 of the p53 gene are amplified in four PCR reactions, which one of the four dNTPs is radiolabeled. The sizes of the PCR products range from 149-249 bp, which are within the ranging of amplification of fairly degraded DNA. For samples that show mutations, we will clone the 2.9 kb PCR fragment encompassing exons 4-9 in to the TA cloning vector (Invitrogen, San Diego, CA) and sequence both strand using M13 and T7 primers. Internal exons (5, 6, 7, 8) will be sequenced with the 5' and 3' intron primers used in the SSCP analysis. The primers employed in the SSCP analysis (34) of the p53 gene are listed below: exon 5-5': TTCCTCTTCCTGCAGTACTC and 5-3' ACCCTGGGCAACAGCCCTGT will produce a 242 bp product; exon 6-5' ACAGGGCTGGTTGCCCAGGGT and 6-3' AGTTGCAAACCAGACCTCAG will produce a 194 bp product; exon 7-5' GTGTTGTCTCCTAGGTTGGC and 7-3' GTCAGAGGCAAGCAGAGGCT will produce a 187 bp product; exon 8-5' TATCCTGAGTAGTGGTAATC and 8-3' AAGTGAATCTGAGGCATAAC will produce a 209 bp product; exon 9-5' GCAGTTATGCCTCAGATTCAC and 9-3' AAGACTTAGTACCTGAAGGGT will produce a 149 bp product; each 10 ml PCR reaction contains: 10 pmol of each primer, 2.5 mM of each dNTP, 1mCi of [a-32p]-dCTP (3000 Ci/mmol), 100 ng of DNA, and 0.02 units of Taq polymerase (Amplitaq, Perkin Elmer/Cetus) under buffer conditions specified by the manufacturer. An aliquot of the PCR sample is diluted (1:25) in 0.1% NaDodSo4, 10 mM EDTA, mixed 1:1 with a sequencing stop solution containing 20 mM NaOH, heated to 95°C for 5 min., chilled on ice, and loaded (total 6 ml) onto a 0.5% MDE polymer (AT Biochem) gel containing 10% glycerol. Following fractionation at 8W for 12-15 hours at room temperature, the gel is dried at 80°C and autoradiographed at room temperature for 4-6 hours. Metal plates are attached to the glass plates during the run to prevent rise in temperature of the p53 gene.

Sequencing of the p53 gene: For the PCR amplification of the 2.9 kb genomic fragment encompassing exon 4-9 the following two primers are used: exon 4-5' GACGGAATTCGTCCCAAGCAATGGATGAT
exon 9-3' GTCAGTCGACCTTAGTACCTGAAGGGTGA. PCR is performed under standard conditions in 100 ml. The amplified 2.9 kb product is cloned into the TA

1000 cloning vector (Invitrogen, San Diego, CA). When paraffin embedded tissues are used, amplification of the entire 2.9 kb genomic fragment is not possible, therefore, the fragment is amplified in three sections: exons 4-5, exons 7-8, and exons 8-9. The T7 or M13 primers are used to sequence the 5' end of exon 4. An additional primer p53 S4-3': TCAGGGCAACTGACCGTGCA allows the sequencing to be done from the 3' end of the exon to yield the complete 264 bases that comprise the exon. The remainder of the exon is sequenced using the same primers used for SSCP analysis .

Analysis plan: Simple summaries and graphical descriptions will be performed of the sample demographics and genetic data. All statistical analyses will be performed using two-sided hypotheses with a significance level of .05. In order to test the primary hypotheses we will perform Fisher's exact tests of the proportion of tumors exhibiting mutations in exon 5 of p53, for postmenopausal vs. premenopausal BC in the Chinese populations and American populations. A multiple logistic regression model will also be developed with menopausal status,family history, and population source (China vs. U.S.) as predictors of mutation in exon 5 among those exhibiting mutations,with a exploration of 2-way interaction terms.

Statistical Power: The primary power calculations are based on the Fisher's exact tests of proportions. Assuming that the mutation distribution is the same in the proposed sample as in the pilot sample, approximately 15% of individuals will exhibit p53 mutations, regardless of age or population origin.Thus we expect a sample of 300 BCs for each of the four age X population groups (post vs. premenopausal and Chinese vs. U.S.) to yield approximately 40 tumors with p53 mutations in each of the 4 subgroups.The pilot data gives us some basis for the calculations, but the sample sizes remain too small to be definitive. We have made every attempt to be conservative in the estimating the effect size of group assignment, so as not to overestimate the power. For the comparison of p53 mutations within the U.S. population group, we assume that the proportion of tumors exhibiting exon 5 mutations will be at least .65 in the postmenopausal group and at most .90 in the premenoupausal group. This gives power of approximately 80% for the test of the main hypothesis. Among the Chinese samples,we believe the power will be considerably higher, as all of the pilot data exon 5 mutations occurred among the postmenopausal women.If the underlying true proportion of exon 5 mutations is less than .35 among the premenopausal women and is greater than .65 among the postmenopausal women, then the power for this comparison will exceed 80%.

Preliminary result of the p53 gene mutation patterns in BC: The

principal investigator of this project, Dr. BaoFa Yu, started this project last year for p53 mutation profiles in premenopausal (less than 35 years of age) versus postmenopausal (more than 70 years of age) BC in both stationary and mixed populations. He hypothesizes that the etiology of BC involves a complex interplay of genetic, hormonal, and dietary factors that are superimposed on the physiological status of the host, and that premenopausal women are more likely to have germ-line mutation in p53 gene, while postmenopausal women will have somatic mutation in p53 gene. Moreover, the p53 gene has specific codons or specific nucleotides mutated that might provide clues to the genetic or environmental factors that contributing to BC. Dr. BaoFa Yu, the PI in this proposal, has found that 3 of 21 BC in postmenopausal women had only mutations in exon 5 of the p53 gene, while the 3 of 21 BC in premenopausal women had mutation in exon 6 and 7 of the p53 gene in the stationary population (Table 1). Dr. Yu and Dr. Sara Sukumar also found that 6 of 40 BC in postmenopausal women had only mutations in exon 5, 6 and 8 of the p53 gene, while the 3 of 21 BC in premenopausal women had only mutations in exon 5 of the p53 gene in the mixed population (Table 2). These results are very exciting because we can see that there is a difference in the patterns of p53 mutations between pre and post menopausal BC, and there is also difference between stationary and mixed populations, it indicates that exon 5 of the p53 gene might be a hot spot area for post-monepausal BC in stationary populations and for premenopausal BC in mixed population. Because of the limited numbers of samples, so we need more resources to continue this project in the further study.

(7)Conclusions: This preliminary results is help to understand why BC incidence in US is 4 to 10 times higher than that in China at the molecular level and compare the incidence between the pre and post menopausal BC and between the stationary population of Shandong, China and mixed population of California, USA. This is related with the etiology of BC such as diet which may be an important factor contributes to informing of BC. BC incidence of women immigrants from China is about 5 time higher than that of women in China. We are also trying to figure out why is so difference. The inactivation of p53 gene function appears to play an important role in human BC. We will use the p53 gene as a gene marker for screening potential patients of BC. Patterns of the p53 gene mutations may give us some clues whether diet, environmental exposure, or genetic factors lead to the higher than normal incidence of BC in the mixed population of California which is useful for the prevention and therapy of BC. Preliminary observations of our laboratory have shown that p53 mutation is often located in exon 5 for postmenopausal BC and located in different exons for premenopausal BC in the stationary Shandong population. In contrast, a p53 mutation is often located in

exon 5 for premenopausal BC and located in different exons for postmenopausal BC in the mixed population of California. These studies emphasize a study gene markers in groups of BC patients, divided into specific categories. This study is focusing on the molecular epidemiology study and is related with the pathogenesis of BC.

In this annual report, Dr. Yu aim to further test our hypothesis #1: pattern of p53 mutations are different between the pre- and post-menopausal BC, and between BC from the stationary and mixed population and #2: mutation in exon 5 are more frequently in FH negative cases, perhaps indicative of sporadic cancer, while exon 6 and 7 may be involved in hereditary cancer. Using DNA extracted from paraffin embedded tumor and normal tissue, Dr. Yu will perform p53 mutation analysis by single stranded conformation polymorphism (SSCP) followed by nucleotide sequencing in a large number of patients samples in order to confirm his hypothesis.

Table 1 Mutation in the p53 gene in young and old groups (42 Cases) of stationary population

Patients No.	Age (years)	FH	SSCP in exon	Shift Condon	Sequence Change	Amino acid substitution
A12	71	-	5	156	CGC to CCC	Arg to Pro
				140	ACC to ATC	
				137	CTG to CCG	
A19	70	-	5	156	CGC to CCC	Arg to Pro
A15	71	-	5	156		
B5	29	+	6	192	CAG to CGG	
B7	33	+	6	195	GTT to GAT	
B13	25	+	7	205	CGT to CAT	

FH:Family History

Table 2 Mutation in the p53 gene in young and old groups (61Cases) of mixed population

Patients No.	Age (years)	FH	SSCP in exon	Shift Condon	Sequence Change	Amino acid substitution
6926	76	-	5	128	CCT to CTT	Pro to Leu
6845	72	-	5	156	CGC to CCC	Arg to Pro
			6	192	CAG to CGG	Gln to Arg
6846	74	-	5	160	ATG to GTG	Met to Val
6912	72	-	8	273	CGT to CAT	Arg to His
5604	80	-	5	162	ATC to ATT	none
6925	72	-	8	269	AGC to SGT	none
				273	CGT to CAT	Arg to His
				289	GAC to GGC	Asn to Gly
6932	30	-	5	154	GGC to GAC	Gly to Asp
6825	35	-	5	147	GTT to GAT	Val to Asp
6646	26	-	5	174	AGG to AAG	Arg to Lys

FH:Family History

(8)References:

1.Sllamon, D.J., Clark, G.M., Wong, S.G., Levin, W. J., et al. 1987: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177-182

2. Prosser, J., Thompson, A. M., Cranston, G and Evans, H.J., 1990: Evidence that p53 behaves as a tumor suppressor gene in sporadic breast tumours. Oncogene 5:1573-1579.

3. Davidoff, A. M., Kerns, B.J. M., Iglehart, J.D. and Marks, J.R., 1991: Maintenance of p53 alterations throughout breast cancer progression. Cancer Res. 51: 2605-2610

4. Sommer, S. S., Cunningham, J., McGovern, R. M., Saitoh, S., Schreder et al, 1992: Pattern of p53 gene mutations in breast cancer of women of the Midwestern United States. J. Natl. Cancer Inst. 84: 246-252.

5. Yu, H., Harris, R.E., Gao, Y. T., et al, 1991: Comparative epidemiology of cancer of the Colon, Rectum, Prostate and Breast in Shanghai, China versus the United States. International Journal of Epidemiology 20(1): 76-81.

6. Willett, W., 1987: The search for the causes of breast and colon cancer. Nature 338:389-394.

7. Henderson, B.E., Ross, R.K., and Pike, M.C., 1991: Toward the primary prevention of cancer. Science 254:1131-1137.

8. Devilee, P., and Cornelisse, C.J., 1991: Genetics of human breast cancer. Cancer Surveys 9: 605-630.

9. Lundberg, C., Skoog, L., Cavenee, W.K., and Nordenskjold, M., 1987: Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13. Proc. Natl. Acad. Sci. USA 84: 2372-2376.

10. Varley, J. M., Brammar, W.J., Lane, D.P., Swallow, J.E., Dolan, C. and Walker, R.A., 1991: Loss of chromosome 17p13 sequences and mutation of p53 in human breast carcinomas. Oncogene 6: 413-421

11. Callahan, R., and Campbell, G., 1989: Mutation in human breast cancer: an overview. J. Natl. Cancer Inst 81:1780-1786.

12. Nigro, J.M., Baker, S.J., Preisinger, A.C., Jessup,, J.M., Hosetetter, R., Cleary, K., Bigner, S.H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F.S., Weston, A., Modali, R., Harris, C..C., And Vogelstein, B, 1989: Mutation in the p53 gene occur in diverse human tumor types. Nature 342:705-708.

13. Bartek, J., Iggo, R., Gannon, J., and Lane, D.P., 1990: Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines. Oncogene 5:893-899.

14. Bartek, J., Bartkova, J., Vojtesek, B., Staskova, Z., Rejthar, A., Kovarik, J., and Lane, D.P., 1990: Patterns of expression of the p53 tumour suppressor in human breast tissues and tumours in situ and in vitro. Int. J. Cancer 46: 839-844.

15. Kovach, J.S., McGovern, R.M., Cassady, J.D., Swanson, S.K, Wold, L.E., Vogelstein, B. and Sommer, S.S., 1991: Direct sequencing from touch preparations of human carcinomas: analysis of p53 mutation in breast carcinomas. J. Natl. Cancer.Inst. (Bethesda) 83: 1004-1009.

16. Osborne, R.J., Merlo, G.R., Mitsudomi, T., Venesio, T., Liscia, D. S., Cppa, A.P.M., Chiba, I., Takahashi, T., Nau, M.M., Callahan, R. and Minna, J.D., 1991: Mutation in the p53 gene in primary human breast cancers. Cancer Res. 51:6194-6198.

17. Runnebaum, I.B., Nagarajan, M., Bowman , M., Soto, D. and Sukumar, S., 1991: Mutation in p53 as potential molecular markers for human breast cancer. Proc. Natl. Acad. Sci. USA 88: 10657-10661.

18. Lane, D. P. and Benchimol, S., 1990: p53 : oncogene or antioncogene? Genees & Dev. 4: 1-8.

19. Vogelstein, B. 1990. A deadly inheritance. Nature (Laond) 348: 681-682

20. Hollstein, M. and Harris, C., 1991: p53 mutation in human cancers. Science (Washington DC) 253: 49-53.

21. Cron de Fromentel, C., and Soussi, T., 1992: TP p53 tumour suppressor gene: a model for investigating human mutagenesis. Genes Chrom. Cancer 4: 1-15.

22. Glickman, B., 1983: Mutation specificity of UV light in E. coli. In: C. W. Lawrence (ed), Induced mutagenesis, pp. 135-177. New York: Plenum Press.

23. Barbacid, M., 1987: Ras Genes. Ann. Rev. Biochem. 56:779-827.

24. IARC Monographs on the the evaluation of carcinogenic risk to humans: Vol. 1-42, Supplement 7, An updating of IARC monographs, pp. 272-310. Lyon, France: International Agency for Research on Cancer, 1987.

25. Coles, C., Condie, A., Chetty, U., Steeel, C.M., Evans, H.J. and Prosser, J., 1992: p53 Mutation in Breast Cancer. Cancer Research 52: 5291-5298.

26. Tsuda, H., Iwaya, K., Fukutomi, T., and Hirohashi, S., 1993: p53 Mutation and c-erbB-2 amplification in intraductal and invasive breast carcinomas of high histologic grade. Jpn. J. Cancer Res. 84: 394-401.

27. Malkin, D., Li, F., Strong, L., Fraumeni, JR., Nellson, C., Kim, D., Kassel, J., Gryka, M., Bischoff, F. Z., Tainsky, M. A., Friend, S. H., 1990: Germ line p53 mutation in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250: 1233-1238.

28. Srivastava, S., Zou, Z., Pirollo, K., Blattner, W., and Chang, E., 1990: Germ line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature. 348:747-749.

29. Anderson, D. E. 1991: Familial versus sporadic breast cancer. Cancer 70(6): 1740-1746.

30. Sidransky, D., Von Eschenbach, A., Rsai, Y.C. et al. 1991: Identification of p53 gene mutations in bladder cancers and utine samples. Science 252:706-709

31. Frye, R.A., BEnz, C.C., Leu, E., Neeld, W.E. And Vogler, W.J. 1986: Detection of amplified oncogenes by differential PCR. Oncogene 4:1153-1157.

32. Sukumar, S., and Barbacid, M. 1990: Specific patterns of oncogene activation in preneoplastic mouse mammary tissues. Oncogene 5: 1271-1277.

33. Dicker, A.P., et al 1989: A rapid method for manual and automated direct sequencing of products generated b PCR. Biotechniques 7: 830.

34. Gaidano, G., Ballerini, P., Gong, J.Z., Inghirami, G., et al. 1991: p53 mutations in human lymphoid malignancies: association with Burkitt's lymphoma and chronic lymphocytic leukemia. Proc. Natl. Acd. Sci. USA 88: 5413-5417.

35. Nakamura, Y.M. et al,1987: Variable number of tandem repeat markers for human gene mapping. Science. 235: 1616-1622.

36. Horn, G. T., Richards, B., Klinger, K.W. 1989: Amplification of a highly polymorphic VNTR segment. Nucleic Acid Res 17: 2140.

37. Oleg K. Glebov, Katherine E. Mckenzie, Christine A. White and Saraswati Sukumar, 1994: Frequent p53 gene mutations and novel alleles in familial breast cancer, 54:3703-3709.

38. Cropp, C.S., Champeme, M_H., Ledereau, R., and Callahan, R. 1993: Identification of three regions on chromosome 17 q in primary human breast carcinomas which are frequently deleted. Cancer Res., 53:5617-5619.

39. Cornelis, R.S., Develee, P., Van Vliet, M., Kuipers-Dijkshoorn, N., Kersenmaeker, A., Bardoel, A., Meera Khan, P., and Cornelisse, C.J., 1993: Allele loss patterns on chromosome 17q in 109 breast carcinomas indicate at least two target regions. Oncogene, 8:781-785.

40. Saito, H., Inazawa, J., Sito, S., Kasumi, F., Koi, S., Sagae, S., KUdo, R., Sito, J., Noda, K., and Nakamura, Y., 1993: Detailed deletion mapping of chromosome 17q in ovarian and breast cancer: 2-cM region on 17q21.3 often and commonly deleted in tumors. Cancer Res., 53:3382-3385.

41.P. Andrew Futreal, Qingyun Liu, Donna Shattuck-Eidens, charles Cochran, Keith Harshman, Sean Tavtigian, L. Michelle Bennett, Astrid Haugen-Strano, Jeff Swensen, Yoshio Miki, Ken Eddingto, Melody McCulre, Chery Frye, Jane Weaver-Feldhaus, Wei Ding, Zahra Gholami, Peter Soderkvist, Lori Terry, Suresh Jhanwar, Andrew Berchuck, J. Dirk Lglehart, Jeff Marks, Dennis g. Ballinger, J. Carl Barrett, Mark H. Skolnick, Alexander Kamb, Roger Wiseman.1994: BRCA1 Mutation in Primary Breast and Ovarian Carcinomas, Science, 266:120-122.

42. Anne M. Bowcock, 1993: Molecular Cloning of BRCA1: a gene for early onset familial breast and ovarian cancer, Breast Cancer Research and Treatment 28:121-135, 1993.

43. Craig S. Cropp, Heli A. Nevanlinna, Seppo Pyrhonen, Ulf-Hakan Stenman, Paul Salmikangas, Hans Albertsen, Ray White, and Robert Callahan, 1995: Evidence for involvement of BRCA1 in sporadic breast carcinomas, Cancer Res., 54:2548-2551.